Title: The differential effect of an activated form of Notch1 in hematopoietic stem cells is related to their fetal versus adult characteristics.

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Foetal hematopoietic stem cells (F-HSCs) are different from adult HSCs (A-HSCs) in terms of gene expression profile, surface markers, differentiation, and self-renewal capacity. F-HSCs maintain their own gene expression and functional profile up to three weeks post natal in mice and then they change and become A-HSC. Although mechanisms of this switch are not clear, it has been demonstrated that imposing the maintenance of a foetal HSC status during adult life results in leukaemia (He S, 2011). It is therefore of importance to understand how regulatory signals act differentially in foetal and adult HSCs.

The Notch signalling pathway plays a critical role in embryonic haematopoiesis. In particular, studies with Notch1 knockout mice have demonstrated that mutant embryos die at embryonic day 10.5 with defects in HSCs generation from hemogenic endothelium. However the effect of activation/inactivation of Notch signaling on F-HSCs is not clear. In adults, Notch activation induces self-renewal and blocks differentiation of HSCs. Studies on the deficiency of Notch signaling in A-HSC diverge from no effect to induction of mieloproliferative diseases (Iannis Aifantis et al, Nature 2011).

Our objective is to elucidate the effect of Notch activation during fetal, newborn and adult stages of HSC.

In this study, HSCs were obtained from transgenic mice for a mild-activated form of Notch1 (NIC) expressed under the regulatory elements of the stem cell leukaemia gene (SCL). The SCL3'Enh construct is active in HSCs (Silberstain, 2005). NIC-WT competitive hematopoietic engraftment assays were performed to determine NIC-HSC functionality at different developmental stages. Also the levels of Notch target genes (Hes1, GATA2 and Notch1), were assessed by quantitative RT- PCR.

Results showed that the activation of Notch signalling was observed in SCL3'EnhNIC adult bone marrow derived A-HSCs, concomitant with a decrease in engraftment potential (41±17%, animals engrafted with NIC-HSCs versus 80±28% engrafted with WT-HSCs, p<0, 06). However, no increment of Notch-targeted genes was observed in NIC expressing F-HSCs and engraftment potential was no different from wild type (75±35% animals engrafted with NIC-HSCs versus 77±38% engrafted with WT-HSCs, p<0,9). Foetal HSC unresponsiveness to Notch activation was also a character observed in 3 weeks postnatal bone marrow. Interestingly, secondary transplantation of F-HSC derived from long-term chimeras showed impaired engraftment suggesting that the mechanisms that regulate Notch activation on HSCs depend on their foetal versus adult developmental stage.

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